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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

ANAEROBIC SOIL METABOLISM STUDIES

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16. Abstract (Limit: 200 words)

This Standard Evaluation Procedure (SEP) describes the procedures used by EPA to evaluate studues on the rate of degradation of pesticide residues in the laboratory under anaerobic conditions in soil. The SEP provides a data reviewer with important guidance in determining the acceptability of an anaerobic soil metabolism study. Details of the proper study design are outlined and the relevance of each aspect of the study design pertinent to pesticide degradation under anaerobic conditions is explained. This information is to be used by the reviewer as guidance in determining study acceptability. The document outlines the general theory of pesticide degradation and provides an overview of the methods used for calculating pesticide degradation rates and half-lives. Important points to consider in assessing the data and in drawing conclusions about the degradation rate of pesticide residues under anaerobic conditions in soil are discussed.

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STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.

Anne L. Barton, Acting Director
Hazard Evaluation Division

TABLE OF CONTENTS

Page

I. INTRODUCTION

A. Objective of the Standard Evaluation Procedure 1
B. General Theory of Terrestrial Field Dissipation .. 1

II. THE SUBMITTED STUDY

A. Purpose 1
B. Study Design 2

III. THE EVALUATION PROCESS

A. Determine the Need for the Study 2
B. Read the Report 2
C. Prepare the Data Evaluation Record (DER) 3
1. Write the Technical Evaluation 3
2. Determine Study Acceptability 5
3. Determine Need for Deferral/Referral to Other HED Branches 5
4. Make a Regulatory Determination 5

IV. APPENDICES

Appendix 1: General Theory 6

Appendix 2: Information to be Included in the Report 9

Appendix 3: Analytical Data to be Submitted by the Registrant 14

Reference Citations 19

STANDARD EVALUATION PROCEDURE FOR ANAEROBIC SOIL METABOLISM STUDIES

I. INTRODUCTION

A. Objective of the Standard Evaluation Procedure

This Standard Evaluation Procedure (SEP) is to be used as an aid for Exposure Assessment Branch data reviewers in their evaluations of the anaerobic soil metabolism studies submitted by registrants in support of pesticide registration.

Anaerobic soil metabolism studies are required by 40 CFR 158.130 in support of registration of an end-use product intended for use on terrestrial food crops (as defined by the Subdivision N Guidelines) and to support registration of a manufacturing-use product which may also be legally used to formulate such an end-use product. Section 162-2 in the Subdivision N Guidelines describes this study and provides a protocol for conducting it[1].

Data on the anaerobic soil metabolism of pesticides are used by the Agency to determine the rate and pattern of pesticide metabolism under anaerobic conditions. This information is used to ascertain effects of flooding or waterlogging on a well aerated soil, a condition which can have an effect on many oxidation-reduction systems that, in turn, may indirectly affect pesticide metabolism and fate.

B. General Theory of Anaerobic Soil Metabolism

The reviewer should be acquainted with the physico-chemical and microbial soil processes causing pesticides to degrade and dissipate in the soil environment. The reviewer is directed to Appendix 1 for a brief description of those soil processes.

II. THE SUBMITTED STUDY

A. Purpose

Anaerobic soil metabolism studies should provide information on (a) the rate of degradation of parent compound in anaerobic soil; (b) the rate of formation and degradation of aerobic degradation products formed during aerobic preincubation; (c) identification of anaerobic soil degradation products; (d) rate of formation and decline of the degradation products; and (e) a material balance. Information gained from this study is directly applied to designing and conducting field dissipation and accumulation studies. For example, it determines the degradation

products one should analyze for in the field soil dissipation studies and to which degradation products rotational crops and nontarget organisms will be exposed.

B. Study Design

The registrant's report should contain (a) a stated goal of the study and (b) sufficient information on the test protocol (compound purity and type of compound used; complete characterization of soils; method used to achieve and maintain anaerobic conditions; proof of anaerobicity of the system; dose level and method of inoculation of soil, sufficient description of sampling frequency) and the analytical protocol (description of methods used for quantitative, qualitative analyses and reports on the quality control procedures used to ensure the validity of the study).

Types of information that the registrant should include in the report is listed in Appendices 2 and 3.

III. THE EVALUATION PROCESS

A. Determine the Need for the Study

The reviewer should initially determine whether the study is required under 40 CFR 158.130. Normally, the study is required to support registration of each active ingredient in pesticide products intended for use on terrestrial food crops and in manufacturing use products intended for use in formulating such end-use products. However, data from an anaerobic soil metabolism need not be submitted if data from an anaerobic aquatic metabolism have been submitted.

B. Read the Report

Reports of anaerobic soil metabolism studies are first reviewed to determine if the following information is present:

1. Are the compound purity and site(s) of radiolabeling specified? If radiolabeling is used, complex molecules must be labeled in appropriate sites to allow each major component of the molecule to be monitored.

2. Is the complete characterization of soil(s) included? Is the soil the same used for the aerobic soil metabolism studies? [pH, moisture capacity, percent organic matter, bulk density, cation exchange capacity, and textural composition (percent sand, silt and clay) and textural class].

3. Are the treatment rate and method of treatment of the soil described? Are the aerobic incubation conditions before the anaerobic conditions have been established well described?

4. Are the methods to achieve and maintain anaerobic conditions well described?

5. Is the proof of anaerobicity presented?

6. Were soil treatments replicated and sampled frequently?

7. Are the methods used to determine material balance described?

8. Are the methods used for quantitative and qualitative analyses of the degraded compound and formation and decline of the degradation products described?

9. Is the material balance determined?

10. Is the half-life estimated?

11. Are the raw data from which a half-life can be calculated included?

The reviewer should determine if there are data gaps within the study. Failure to provide adequate information may be sufficient for the reviewer to reject the study. In cases where considerable information is missing, the reviewer should make a detailed list of the deficiencies and omissions and not provide a detailed scientific review of the report at this time.

C. Prepare the Data Evaluation Record (DER)

After the reviewer has determined there are no data gaps which would cause the report to be rejected, the reviewer prepares a DER according to the Standard Format For Preparation of Environmental Fate Reviews.

1. Write the Technical Evaluation.

The reviewer should use Appendices 2 and 3 as aids in this process. The technical evaluation should be prepared with the following points in mind:

a. The test protocol should provide adequate information as to how and under what conditions the study was conducted. Consult Appendix 2.

b. The analytical protocol should provide information on sensitivity and specificity of the analytical method(s) used and indicate how the results were determined. Consult Appendix 3.

c. The material balance aids in determining the validity of the study. Is all or the major portion (90-95%) of the test chemical applied to the soil accounted for?

d. The rate of degradation of the test material provides a half-life estimate of the parent compound. It answers the the following questions:

- Does the test material or the aerobic degradation product(s) developed during aerobic incubation degrade?
- Is it persistent in the anaerobic soil environment?
- Is it more or less stable under anaerobic conditions than under aerobic conditions?

It also provides a measure of predicting the degradation rate under anaerobic field conditions.

e. It is essential that the report provide the identity of the soil degradation products of the test substance in order to answer the following questions:

- To what does the test chemical or its aerobic degradation product(s) degrade?
- How extensive is degradation? Does it mineralize to inorganic compounds, e.g. CO_2?
- What compounds can be expected to occur and should be looked for in the field dissipation studies?
- To what compounds will rotational crops and other non-target organisms (e.g., aquatic organisms) be exposed?

f. The rate of formation and decline of degradation products provides a half-life estimate for the degradation products. This answers the following questions:

- Are the degradation products persistent in the soil environment and thereby available for leaching to the water table?
- At what rate do they decline?
- How does the rate compare with that under aerobic conditions?

g. Quality control information provides assurance of the integrity of the study. This information answers the following questions:

- Were logbooks and record-keeping procedures maintained?
- Were quality procedures maintained during sampling, storage and analysis of soil samples. Were good laboratory practices followed?
- Was the soil for test maintained in a manner to maintain viable microbial populations?

h. A degradation scheme of the test compound and degradation products can suggest what mechanisms contributed to the degradation.

2. Determine Study Acceptability.

Were the stated goals of the study appropriate and clearly defined? Was the study conducted in a scientifically sound manner to accomplish those goals? If so, the reviewer then determines the acceptability of the study and considers whether the study, as it stands alone or in light of other studies (combined testing, surrogate studies, or waivers), supports the requested registration action.

If the study is deficient, a detailed description of the deficiencies and recommendations on how to rectify the deficiencies is prepared and included in the DER.

3. Determine Need for Deferral/Referral to Other HED Branches.

If the reviewer concludes either that (a) the test chemical or its degradates have a potential for reaching ground water due to their persistence, (b) residues may occur in rotational crops due to their persistence (c) that there may be exposure to other non-target organisms due to persistence, then the Toxicology, Residue Chemistry and/or Ecological Effects Branches may have to be notified. Final decision on the need for deferral/referral is to be made after the evaluation of the other environmental fate studies.

4. Make Regulatory Determination.

Based on the technical evaluation, the reviewer determines whether the study satisfies the data requirement for an anaerobic soil metabolism study as listed in 40 CFR 158.130.

APPENDIX 1

General Theory

Pesticide products reaching the soil may either decompose completely to inorganic compounds (e.g., CO_2), be altered in varying degrees and become part of the soil complex or remain intact in the soil ecosystem. In all cases, residues may be available for uptake by rotational crops and exposure to non-target organisms. Degradation products may become important when their rate of formation exceeds their rate of decomposition.

Numerous factors are known to influence fate and behavior of pesticides in the soil system. Many pesticides are partially degraded in soil by non-biological processes and some reactions can be brought about by either microbial enzymes or by strictly chemical reaction. Usually these two processes work in conjunction [2,3]. Products of partial chemical degradation, however, will depend on the microorganisms for further degradation. The most complex process by which pesticides are degraded in soils involves microbial utilization of the pesticide as an energy source[4]. However, it appears that microbial degradation of most agricultural chemicals applied to soil at low concentrations occurs primarily via cometabolism by soil microorganisms using soil organic matter as their main source of energy[6].

While the aerobic soil metabolism study is designed to measure degradation of the test compound from biological degradation due to microorganisms in the soil complex under ordinary aerobic soil conditions in addition to the soil-associated physicochemical processes, the anaerobic soil metabolism study is to measure degradation of test compound and its aerobic soil metabolites due to microorganisms under anaerobic conditions which can occur by flooding or water-logging on a well-aerated soil.

Microorganisms comprise a large fraction of the living biomass in soil--up to 80% when soil algae are included even though they are not all active under the same conditions. Microbial degradation in soil has been viewed as a process in which organisms adapt to the chemical and use it as a preferred food source[6]. The reader is directed to the Standard Evaluation Procedures for Aerobic Soil Metabolism Studies for the general theory of soil metabolism of pesticides.

Microbes vary considerably in their responses to change in concentrations of gases in the soil atmosphere as well as pH, organic matter, soil moisture contents, temperature and cation exchange capacity [7]. Some bacteria are strictly anaerobic and are unable to grow in the presence of oxygen while others

are strictly aerobic. Fungi are also strict aerobes, and as in other groups, the quantitative relationships between growth and oxygen supply varies in different species[8].

In a matter of hours of flooding the soil, the oxygen supply may be consumed and anaerobic organisms take precedence[9]. Anaerobic conditions often lead to the accumulation of sulfides and ferrous ion, with a decrease of nitrate and available phosphates. The redox-potential drops. Since the oxygen is not present to serve as an electron acceptor, other inorganic substances must serve in this capacity. Two of the most striking effects are the reduction of iron compounds and of nitrates[9]. The ferric iron oxides and hydroxides have a low solubility but the ferrous forms are very soluble; and, thus, anaerobic conditions with sufficient energy supply will cause high solution concentration of ferrous ion. This should be of importance in the reaction of some soil additives. Nitrates are reduced to several nitrogen gases (NO, NO_2 and N_2) which are then lost to the system. Anaerobic decomposition of organic substrates also produces volatile fatty acids and other organic acids.

When an organic additive is decomposed by one or only a few species of organisms and these are either aerobic or anaerobic, then, an anaerobic environment will either stop or accentuate the decomposition. The change from aerobic to anaerobic occurs at about an oxygen concentration of 3×10^{-6} M[10]. Larger soil granules about 3 mm or more in radius and having small pores are water saturated much of the time; and, therefore, the interior of these granules may be anaerobic even in an otherwise aerobic soil, because of the low solubility and diffusion of O_2 in water. Thus, it is probable that anaerobic micro-environments occur in most soils allowing the growth of anaerobic bacteria which can fix nitrogen.

Soil microorganisms can decompose most synthetic organic soil additives as well as the natural soil organics, but there are some limitations. Some synthetic organic chemicals involve unusual molecular structures to which organisms may not be well adapted[11]. It has been reported that chlorinated hydrocarbons (e.g. DDT) are degraded more rapidly under anaerobic conditions than under aerobic conditions[12].

Therefore, the soil metabolism of pesticides should be studied under the anaerobic conditions in addition to under the aerobic conditions.

As non-biological soil degradation processes, there are numerous soil-associated physico-chemical reactions. Hydroxylation and oxidation are common soil chemical reactions[4]. Less is known of other mechanisms (reduction, isomerization, and free-radical reactions). All the soil associated chemical reactions are initiated through water acting as the medium of reaction or

reactant or as both. These chemical reactions may be catalized by clay, metal oxides, metal ions, or extracellular enzymes[4]. The organic matter fraction of soil can play a major role in the chemical degradation of pesticides in providing reactive functional groups[13,14] as well as by retaining the molecule (adsorption) which allows these processes to take place[15].

Both biodegradation and soil physico-chemical degradation are affected by soil pH. For example, microbial oxidation is most rapid between pH 6 and pH 8[14]. Soil water is seldom neutral and its pH range may vary from strongly acidic (pH 3) to strongly basic (pH 10.5)[16]. A number of pesticides will hydrolyze rapidly under aqueous acidic and alkaline conditions that can be found in soil. For further information see the discussion in the Standard Evaluation Procedures for Hydrolysis Studies.

APPENDIX 2

Information to be Included in the Report

Section 162-2 in the Subdivision N Guidelines[1] describes this study and gives a protocol for conducting this study. Also, Section 160.5 in the Subdivision N Guidelines[1] describes general reporting and evaluation requirements for this study.

A. Information To Be Included

1. Dates on which the study began and ended;

2. Name and address of the laboratory or institution performing the test;

3. Location where the test was performed;

4. Names of the principal investigators;

5. Signatures of each of the senior personnel responsible for the study;

6. Certification by the registrant that the report is a complete and unaltered copy of the report provided by the testing facility.

7. The report should identify the test substance and include the chemical name and purity of active ingredient, molecular structure, manufacturer and lot and sample number(s) of the test substance(s), properties of the test substance(s) [including physical state, pH] if not reported elsewhere.

8. Description of the soil source and soil characteristics;

9. A detailed report in tabular form of the residue data from the treated soil. Graphs are often included to expand on the data given in tabular form.

10. A complete discussion of the results of the study which should include a discussion of the following:

   a. Material balance;

   b. Degradation rate and half-life estimate of the parent;

   c. Rate of formation and decline of the degradation products;

   d. Identity of residues occurring at levels of 0.01 ppm or greater;

e. Residue decline curves.

B. Detailed Discussion

1. The test substance.

The chemical to be tested must be chemically pure. The use of radiolabeled compounds is preferred. Carbon-14 is usually used; ring labeling is preferred to side-chain labeling since the radioactivity is usually less easily removed from the central ring positions. If the molecule is complex and consists of moieties that are easily separated, separate studies using compounds labeled at different positions may be necessary. Tritium labeled compound may also be used if proton exchange does not interfere with the experiment.

Use of radiolabeled chemicals rather than non-labeled ones allows: (a) the degradation process to be followed at a much lower concentration and (b) easier determination of mass (material) balance. If non-radiolabeled compound is used, the study must be conducted with the technical or purer grade of the active ingredient(s).

Sometimes, initial concentrations of the test substance in soil exceeding the proposed use rate should be studied to permit measurement of the disappearance of the parent compound and identification of the major degradates formed.

2. The test soil.

Since the anaerobic soil incubation is preceded by the aerobic soil incubation of 30 days or one half-life (whichever is shorter), the soil used in this study should be the same one selected for testing in the aerobic soil metabolism study.

The rate, type and degree of metabolism of the pesticide and its major degradates should be determined in a sandy loam or silt loam. A different soil may be used if representative of the soil at the intended sites. For example, celery is usually grown in a muck soil. Thus, to support such a use it may be necessary to conduct the study in muck soil. If the use covers a crop that is grown in various soil types with wide geographic distribution, then more than one soil type must be studied. For example, soybeans are grown in large geographical areas encompassing several distinct soil types. Here, it will be necessary to conduct the study in both a sandy loam and a loamy clay soil.

There is no best way to collect and store soils for conducting this study[17]. The soil should be a collection of subsamples from the top layer of the field soil. The majority of the field

microbial populations is located in the top layer of soil (the upper 14 centimeters)[18]. This is the area where nutrient levels and oxygen availability is high[19]. The soil should not have received prior pesticide applications. If soil has received prior treatment of the same or similar type of compound, then the microbial enrichment or adaptation to that type of compound may have occurred. Subsequent pesticide applications may then degrade more rapidly[16].

As in most routine soil analyses[20], the soil should be sieved through a 2 mm sieve. This removes extraneous debris which could adsorb the test compound thus affecting the results of the study. The soil should preferably be freshly acquired from a typical use area or have been maintained in such a manner that viable microbial populations are present in the soil. The soil should be stored in a clean, chemical-free environment. The soil may be dried to approximately 75% of the moisture level at 1/3 bar (Note: 1/3 (0.33) bar is a laboratory measure of the soil moisture at field capacity-the amount of water remaining in soil after excess water has been removed by force of gravity[17]). However, it is important the soil not become air-dry as that can adversely affect the microorganism populations present[17].

Complete information on the soil class, textural characterization of the soil, soil pH, and % organic matter is necessary to verify that the soil is representative of agricultural soils. Use of U. S. soils from typical use areas is preferred but if foreign soils are used, such data on soil class and textural classification are needed to indicate its similarity to U. S. agricultural soils from typical use areas. When foreign soils are used, additional studies conducted with U.S. soils and of sufficient duration will also be needed to provide assurance that any possible differences in microbial populations between the foreign and U.S. soils does not result in different patterns of degradation.

Since many reactions are pH sensitive, the nature of the soil pH reaction is an important consideration. The organic matter content is a measure of microbial viability, being the main energy source for soil microorganisms.

3. Test procedures.

A sample of the soil treated with one or more concentrations of the test substance and incubated for 30 days or one half-life (which ever is shorter) should be converted from aerobic to anaerobic conditions by either water-logging or purging the soil with inert gases. The reader is directed to the Standard Evaluation Procedures for Aerobic Soil Metabolism for aerobic incubation. Where organic content of the soil is deficient, organic amendment should be supplied to the treated soil within 30 days to ensure a substance for anaerobic conditions.

a. Proof of anaerobicity

The proof of anaerobicity should be provided. It is probably the best to measure the redox potential to determine the condition of test system(21). It was reported that the redox potential of most of the flooded soils reached negative values(22).

b. Treatment rate.

The concentration of the test substance should be at levels sufficient to permit measurement of the disappearance of parent compound and identification of major degradation products. The dose should be at the highest proposed field use rate. Separate studies at higher doses (5 to 10X) may be necessary for identification of degradation products.

c. Study conditions.

The study conditions are the same as in the aerobic soil metabolism studies except anaerobic conditions. For aerobic preincubation, treated soil should be maintained at a constant temperature between 18 and 30°C that is most representative of the use area. The recommended soil moisture content is at a level of 75 percent of 0.33 bar moisture. These are the soil moisture conditions most conducive to metabolism by aerobic microorganisms and is a compromise of the unlimited variety of combinations of temperature and moisture levels found in the natural environment.

One hundred percent of one-third (0.33) bar soil moisture represents the upper limit of soil moisture for aerobic microbial activity (equivalent to field moisture capacity). The 75% of 0.33 bar is a safe moisture level still within the optimum range[17]. It is probably a moisture level more prevalent in soil than 100% of 0.33 bar. If soil is maintained at another soil moisture level, this level should also be reported relative to the theoretical moisture content at 75% of 0.33 bar. However, the soil moisture level should be within 10-12% of the level at 75% of 0.33 bar moisture.

Soil samples should be taken and replicated sufficiently to provide interpretable data for kinetic analysis. The soil study should include untreated controls. Soil samples taken just prior to the anaerobic conversion provide zero time data for anaerobic soil metabolism. Where organic content of the soil is deficient, organic amendment should be supplied to the treated soil within 30 days to ensure a substrate for anaerobic microorganisms.

d. Duration of study

The length of study need not extend beyond 60 days.

e. Sample collection

Guidelines[1] recommend that preferred sampling intervals are 30 and 60 days after anaerobic conditions have been established. However, it will be beneficial to obtain more data points (within 60 days) for the estimation of half-lives, although it is not impossible to compare the degradation under anaerobic conditions to that under aerobic conditions using the data obtained from the 30 and 60 day samplings. In addition, sampling should be done at the end of aerobic incubation as mentioned earlier.

If trapping solutions are used, they should be sampled when the soil is sampled.

Soil samples should be analyzed as soon as possible after being taken. If samples are stored before extraction and analysis, it must be shown that the pesticide residues will not degrade under the storage conditions. Spiked samples should be maintained under identical conditions and extracted with treated samples unless preliminary data are available which show that additional degradation does not occur under the storage conditions. The following conditons are considered acceptable for storage: freezing samples at $\leq$ 0 °C as soon after collection as possible until analyzed.

APPENDIX 3

Analytical Data to be Submitted by the Registrant

A. Analytical Procedures

1. Analytical methods.

A full description of the analytical methods used in all steps of the analytical protocol must be submitted, including the following information:

a. Name (and signature), title, organization, address and telephone number of the person(s) responsible for the planning and supervision/monitoring and laboratory procedures/analyses;

b. Analytical method(s) title/designation/date

c. Source of analytical method(s) [e.g., Pesticide Analytical Manual (P.A.M), Vol. II, scientific literature, Company reports);

d. Principles of the analytical procedure (description);

e. Copy of the analytical method(s) detailing in stepwise fashion the procedures (extraction, clean-up, derivatization, determination, calculation of the magnitude of the residue);

f. Reagents or procedural steps requiring special precautions (to avoid safety or health hazards, explain);

g. Identification of the chemical species determined;

h. Describe modifications, if any, of the analytical method(s);

i. Extraction efficiency;

j. Instrumentation [make/model, type/specificity of detectors, column(s) packing materials, size, gas carrier, flow rates, temperatures, limit of detection and sensitivity, calibration procedures, etc].

k. Interference(s), if any;

l. Confirmatory techniques [e.g., other column packings, detectors, etc.]

m. Date(s) sample taking, extraction and residue analyses;

n. Sample identification [Coding and labeling information];

o. Residue results [raw data, laboratory worksheets, stepwise calculation of residue levels, dilution factors, peak heights/areas, method correction factors applied (e.g., storage stability and method validation recovery values, standard curve(s) used, ppm found of "total" residues and of individual components if of special concern, range of residue values, representative chromatograms, spectra of control and treated samples);

p. Statistical treatments (of raw data);

q. Other [any and all additional information the registrant/researcher considers appropriate and relevant to provide a complete and thorough description of residue analytical methodology and the means of calculating the residue results].

B. Method Validation

A full description of the method recovery validation procedures must be submitted and include information on the following: the recovery level(s) of the test compounds from the soil (substrate) at various fortification level(s) using the residue analytical methodology; a validated method sensitivity level; results of the study and statistical tests applied, a stepwise presentation of the procedure for calculating percent recovery from the raw data; all the data/information necessary to independently verify the results; summary of data results; and conclusions drawn from the data results.

C. Quality Assurance

A complete description of the measures taken to ensure the integrity of the test and analytical protocols should include information on the following: logbooks and/or record-keeping procedures; representative instrument printouts (chromatograms, spectra, etc.); sample coding; use of replicate samples and control blanks; use of written and validated analytical methodology for residue analyses, including modification(s) made; skilled laboratory personnel; well-equipped laboratory facility; use of high quality glassware, solvents, and test compounds; minimal contamination; calibration and maintenance of instruments; good laboratory practices in handling the test substance(s).

D. Residue Analysis

1. Samples should be subdivided and analyzed as follows:

a. Total residues in flooded water.

If the anaerobic conditions are achieved by water-logging, the soil and water are separated and the residues in water and soil are determined. When ^{14}C labeled parent compound is used, the water sample will be radioassayed by LSC.

b. Total residues in soil.

The soil will be combusted in an oxidizer and CO_2 formed will be trapped in appropriate solvents and radioassayed by LSC.

c. Total residues in volatile traps.

Radioactivity in volatile traps (for CO_2 and other volatile organic compounds) is measured. The above three provide a preliminary material balance.

d. Organic solvent extractable aqueous residues.

The water sample is partitioned in appropriate organic solvents and the radioactivity in organic solvents and aqueous phase is measured.

e. Extractable soil residues

Soil is extracted with appropriate solvents, filtered, and reextracted if necessary. Exhaustive extraction methods are sometimes necessary when residues are not extracted by gentler means. The reviewer should determine that an appropriate extracting solvent was used in the study. The reader is directed to Chesters, et al.[23] for a general overview on the subject of pesticide extraction and analysis of soil without elaboration on specific procedures and to Hance and McKone[24] for specific procedures for herbicides.

Extractable residues are considered as those being immediately available for uptake by rotational crops, exposure to non-target organisms and for leaching into the ground water. They are also available for further degradation.

f. Non-extractable soil residues

The post-extracted soil is combusted in an oxidizer and the CO_2 formed is trapped in appropriate solvent. Non-extractable residues are those that may, at some later time, become available for uptake by rotational crops or for exposure to non-target organisms. They are also a measure of the soil binding potential of the test chemical and degradation products.

The sum of the total volatile residues, the total soil extractable residues, the non-extractable soil residues and the total residues in flooded water (expressed as percent of the total applied) make up the material balance.

The material balance summed should ideally equal to 100% of the amount of test chemical applied. Thus, the material balance is a factor in determining the validity of the study. Studies wherein the material balance does not account for a large

percentage (85-95%) or accounts for greater than 105-115% of the amount of test chemical applied may be invalid and not useful in satisfying the data requirement.

2. Identification of metabolites

The Guidelines[1] require that residues occurring at a level of 0.01 ppm or greater at normal field application rates under the label treatment schedule should be identified when feasible. Thin-layer chromatography (TLC) of extracted residues with co-chromatography of known standards is adequate for tentative qualitative identification. Autoradiography of TLC plates provides a record for locating any labeled degradation products. Liquid chromatography (LC) or gas chromatography and mass spectroscopy (GLC/MS) analysis provides positive metabolite identification. Quantitative analysis can be either by LSC, LC or GLC. All analytical methods must be examined for their specificity, sensitivity and recovery.

Identification of metabolites is a major part of the soil metabolism study and involves most of the analytical resources. Metabolites found in the anaerobic soil metabolism study may be found in the field conditions. Knowing the identity of the degradation products aids in preparing a degradation scheme of the test chemical and analyzing the field soil samples.

3. Half-life estimate

As mentioned earlier, the reaction kinetics of pesticide degradation in soil are concerned primarily with the decline in concentration of the pesticide with time. For pesticides that are not strongly sorbed to soil, rates of degradation approach first-order kinetics at low concentrations and zero-order at high concentrations. Since pesticides are usually applied to soil at low concentrations and are often strongly sorbed, the rates of most chemicals should approach first-order kinetics. That is, the concentration of the pesticide in soil (which is usually low compared to the other reacting materials in soil) determines the velocity of the reaction[25]. However, rates tend to decrease with time more than would be expected which suggest sorptive forces are still active in the soil[6]. The rate of loss is proportional to its concentration in the soil and can be expressed by the equation:

$$-\frac{dc}{dt} = Kc^n \qquad (1)$$

where c = the concentration of the pesticide, K = the rate constant, t = time and n is the order of the reaction. For first order kinetics, n = 1, therefore,

$$-\frac{dc}{dt} = Kc \qquad (2)$$

where C is the amount of pesticide present in soil at time t. Here, the first-order reaction means that the disappearance of the pesticide is proportional to the amount left in the soil.

The first order is most commonly used since it is the simplest to use. Assuming first-order kinetics, the pesticide's half-life (the time required for the concentration to become equal to one-half of the original concentration) can be calculated. The dissipation of most pesticides from soils can be considered as following a first-order reaction[26] at least over a portion of the degradation curve[4,11]. It is reported that, overall, the reaction kinetics involved in decomposition of pesticides follow mostly the zero-, first-, and second-order reaction systems[27]. It is doubtful that any one single rate equation will ever be found which is applicable to all or most pesticides in soil[4]. The half-life estimate based on the kinetic analysis of the data generated in the aerobic metabolism study becomes a measure of persistence of pesticides under field conditions. However, it should be noted[25] that data summarized[11] show that the relationships imply that the proportion of the chemical degraded with time decreases as the concentration of the chemical decreases. The rate at which the last traces of a chemical disappears can be very slow[25].

Although the half-life estimate from the anaerobic soil metabolism data seems inadequate because the study is designed to last only 60 days and not enough data points are taken, it provides a measure to compare the degradation rate under anaerobic conditions, which can occur in the field after the pesticide has been applied, with that under ordinary aerobic conditions.

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